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1   MVCGGFACSKNCLCALNLLYTLVSLLLIGIAAWGIGFGLI  HT4P
1   MVCGGFACSKNALCALNVVYMLVSLLLIGVAAWGKGLGLV  g457937
1   MVCGGFACSKNALCALNVVYMLVGLLLIGVAAWAKGLGLV  g971980

41  SSLRVVGVVIAVGIFLFLIALVGLIGAVKHHQVLLFFYMI  HT4P
41  SSIHIIGGVIAVGVFLLLIAVAGLVGAVNHHQVLLFFYMI  g457937
41  SSIHIIGGVIAVGVFLLLIAVAGLVGAVNHHQVLLFFYMI  g971980

81  ILLLVFIVQFSVSCACLALNQEQQGQLLEVGW-NNTASAR  HT4P
81  ILGLVFIFQFVISCSCLAINRSKQTDVINASWWVMSNKTR  g457937
81  ILGLVFIFQFGISCSCLAINLSKQAGIIN             g971980

120 NDIQRNLNCCGFRSVNP-----NDTCLASCVKSDHSCSPC  HT4P
121 DELERSFDCCGLFNLTTLYQQDYDFCTAICKSQSPTCQMC  g457937
109                                           g971980

155 APIIGEYAGEVLRFVGGIGLFFSFTEILGVWLTYRYRNQK  HT4P
161 GEKFLKHSDEALKILGGVGLFFSFTEILGVWLAMRFRNQK  g457937
109                                           g971980

195 DPRANPSAFL                                HT4P
201 DPRANPSAFL                                g457937
109                                           g971980
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(57) Abstract

The present invention provides a human transmembrane 4 protein (HT4P) and polynucleotides which encode HT4P. The invention also provides expression vectors, host cells, agonists, antisense molecules, antibodies, or antagonists. The invention also provides methods for treating disorders associated with expression of HT4P.

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NEW HUMAN TRANSMEMBRANE PROTEIN

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of a new transmembrane protein and to the use of these sequences in the diagnosis, prevention, and treatment of cancer, neurological disorders, and smooth muscle disorders.

BACKGROUND ART

Membrane proteins are divided into two groups based upon the ease with which the proteins can be removed from the membrane. Extrinsic or peripheral membrane proteins can be removed using extremes of ionic strength or pH, or other disruptors of protein interactions. Intrinsic or integral membrane proteins are released only when the lipid bilayer of the membrane is dissolved by detergent.

The majority of known integral membrane proteins are transmembrane proteins which contain extracellular, transmembrane, and intracellular domains. Transmembrane proteins are typically embedded in a cell membrane by one or more regions comprising 15 to 25 hydrophobic amino acids that adopt an α-helical conformation. Transmembrane proteins are classified as bitopic (Types I and II) and polytopic (Types III and IV). While bitopic proteins span the membrane only once, polytopic proteins span the membrane multiple times and type III proteins have multiple stretches of hydrophobic residues (Singer, S.J. (1990) Annu. Rev. Cell Biol. 6:247-96). Transmembrane proteins carry out a variety of important cellular functions including signal transduction (e.g., growth factor receptors) and transport of ions or metabolites (e.g., ion channels).

Recently a multigene family encoding type III integral membrane proteins was identified (Wright, M.D. and Tomlinson, M.G. (1994) Immunol. Today 15:588-94). The transmembrane 4 superfamily (TM4SF) or tetraspan family traverse the cell membrane four times. TM4SF proteins are found predominantly in cells of hematopoietic origin and in tumors and include a number of platelet and endothelial cell membrane proteins, CD9 (lung adenocarcinoma antigen MRP-1), the platelet and melanoma-associated antigen CD63, leukocyte surface glycoproteins, CD53, CD37, CD63, and R2, the tumor associated antigen TAPA-1 (CD81), the colonal carcinoma antigen CO-029, mink lung epithelial protein TI-1, the tumor-associated antigens L6 and SAS ,and a gene amplified in human sarcomas (Wright and Tomlinson, supra; Jankowski, S.A. et al. (1994) Oncogene 9:1205-11). The proteins share 25-30% amino acid sequence identity.

The predicted structure of the TM4SF proteins reveals a topology where the N- and C-

termini are intracellular and the major hydrophilic domain, located between transmembrane domains 3 and 4, is extracellular. Most of these proteins have N-linked glycosylation sites within the hydrophilic domain. TM4SF proteins are most conserved in their transmembrane and cytoplasmic domains and most divergent in their hydrophilic extracellular domains. The high level of conservation seen in the transmembrane and cytoplasmic domains suggest an effector/signaling function. The divergence of the extracellular domains suggests that these hydrophilic domains provide functions specific to each protein such as ligand binding or protein-protein interaction (Wright and Tomlinson, supra).

A number of TM4SF proteins have been implicated in signal transduction, control of cell adhesion, regulation of cell growth and proliferation, including development, oncogenesis, motility, and the ability to suppress metastatic potential. Expression of a number of TM4SF proteins is associated with a variety of tumors and is altered when cells are growing or activated. For example, CD9, CD53 and CD82 are upregulated when lymphocytes are activated and cell surface expression of CD37 is lost when B cells are activated. Other TM4SF proteins are implicated in cell growth due to their association with tumor cells. Although CD9 is not expressed on resting B and T lymphocytes, it is a marker for 90% of non-T acute lymphoblastic leukemia cells and for 50% of acute myeloid and chronic lymphoid leukemias. Anti-CD9 antibodies inhibit the motility of a variety of cancer cell lines and inhibit the metastatic potential of the mouse BL6 cell line (Miyake, M. and Hakomori, S. (1991) Biochem. 30:3328-34). Expression of CD9 in transfection experiments correlated with suppression of metastatic potential and cell motility (Ikeyama, S. et al. (1993) J. Exp. Med. 177:1231-37). Similarly CD63 is not expressed on normal tissue melanocytes, but it is expressed in early stage melanoma. The gene encoding SAS (sarcoma amplified sequence) is amplified in a subset of human sarcomas. The chromosome encoding this gene, 12q13-14, has previously been found to contain a number of growth-related genes including the GLI proto-oncogene and MDM2, a modulator of the tumor suppressor gene, p53 (Jankowski et al, supra).

The discovery of a new transmembrane 4 protein and the polynucleotides encoding it provides additional tools for studying the function of TM4SF proteins in signal transduction and cell growth control under normal and disease conditions. A new TM4SF satisfies a need in the art by providing new diagnostic or therapeutic compositions useful in the treatment or prevention of cancer, neurological disorders, and smooth muscle disorders.

DISCLOSURE OF THE INVENTION

The present invention features a new human transmembrane 4 protein hereinafter

designated HT4P and characterized as having similarity to other transmembrane 4 proteins.

Accordingly, the invention features a substantially purified HT4P having the amino acid sequence shown in SEQ ID NO:1.

One aspect of the invention features isolated and substantially purified polynucleotides that encode HT4P. In a particular aspect, the polynucleotide is the nucleotide sequence of SEQ ID NO:2.

The invention also relates to a polynucleotide sequence comprising the complement of SEQ ID NO:2 or variants thereof. In addition, the invention features polynucleotide sequences which hybridize under stringent conditions to SEQ ID NO:2.

The invention additionally features fragments of the polynuclceotides encoding HT4P, expression vectors and host cells comprising polynucleotides that encode HT4P and a method for producing HT4P using the vectors and host cells. The present invention also features antibodies which bind specifically to HT4P, and pharmaceutical compositions comprising substantially purified HT4P. The invention also features agonists and antagonists of HT4P. The invention also provides methods for treating disorders associated with expression of HT4P by administration of HT4P and methods for detection of polynucleotides encoding a transmembrane 4 protein in a biological sample.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A, 1B and 1C, show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of HT4P. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

Figure 2 shows the amino acid sequence alignments among HT4P (SEQ ID NO:1), a human SAS member of TM4SF (GI 457937; SEQ ID NO:3), and a pig SAS (GI 971980; SEQ ID NO:4). The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 3A, 3B, and 3C show the hydrophobicity plots (DNASTAR™) for HT4P, SEQ ID NO:1; human SAS, SEQ ID NO:3; and pig SAS (SEQ ID NO:4). The positive X axis reflects amino acid position, and the positive Y axis, hydrophobicity.

MODES FOR CARRYING OUT THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to

limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"Peptide nucleic acid", as used herein, refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63).

HT4P, as used herein, refers to the amino acid sequences of substantially purified HT4P obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or

recombinant.

"Consensus", as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, or which has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte clone using the GELVIEW™ Fragment Assembly system (GCG, Madison, WI), or which has been both extended and assembled.

A "variant" of HT4P, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HT4P, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "agonist", as used herein, refers to a molecule which, when bound to HT4P, causes a change in HT4P which modulates the activity of HT4P. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to HT4P.

The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to HT4P, blocks or modulates the biological or immunological activity of HT4P. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other

molecules which bind to HT4P.

The term "modulate", as used herein, refers to a change or an alteration in the biological activity of HT4P. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological properties of HT4P.

The term "mimetic", as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of HT4P or portions thereof and, as such, is able to effect some or all of the actions of serine protease TM4SF-like molecules.

The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding HT4P or the encoded HT4P. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide which retains essential biological characteristics of the natural molecule.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

"Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

The term "hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen binds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for in situ hybridization).

The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, for the sequence "A-G-T" binds to the complementary sequence "T-C-A".

Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "stringent conditions", as used herein, is the "stringency" which occurs within a range from about Tm-5°C (5°C below the melting temperature (Tm) of the probe) to about 20°C to 25°C below Tm. As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The term "antisense", as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "portion", as used herein, with regard to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein "comprising at least a portion of the amino acid sequence of SEQ ID NO:1" encompasses the full-length human HT4P and fragments thereof.

"Transformation", as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "antigenic determinant", as used herein, refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon

the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "sample", as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding HT4P or fragments thereof may comprise a cell, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), an extract from cells or a tissue, and the like.

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding HT4P in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

"Alterations" in the polynucleotide of SEQ ID NO:2, as used herein, comprise any alteration in the sequence of polynucleotides encoding HT4P including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes HT4P (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to SEQ ID NO:2), the inability of a selected fragment of SEQ ID NO:2 to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HT4P (e.g., using fluorescent in situ hybridization [FISH] to metaphase chromosomes spreads).

As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fa, $F(ab')_2$, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind HT4P polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

THE INVENTION

The invention is based on the discovery of a new human transmembrane 4 protein (HT4P), the polynucleotides encoding HT4P, and the use of these compositions for the diagnosis, prevention, or treatment of cancer, neurological disorders, and smooth muscle disorders.

Nucleic acids encoding the human HT4P of the present invention were first identified in Incyte Clone 2279874 from the normal prostate cDNA library (PROSNON01) through a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 638873/ BRSTNOT03, 1418260/ KIDNNOT09, 1444655/ THYRNOT03, 2228841/ PROSNOT16, 2243238/ PANCTUT02, 2279874/ PROSNON01, and 2726714/ OVARTUT05.

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1 as shown in Figures 1A, 1B and 1C. HT4P is 204 amino acids in length and has potential N-linked glycosylation sites at N113 and N137. Cysteine residues at C3, C8, C12, C14, C94, C96, C128, C129, C140, C144, C151, and C154 represent potential disulfide bridging sites. A potential casein kinase II phosphorylation site is located at S177, and potential protein kinase C phosphorylation sites are located at S42, S117, and T187. As shown in Figure 2, HT4P has chemical and structural homology with human SAS (GI 457937; SEQ ID NO:3) and pig SAS (GI 971980; SEQ ID NO:4). In particular, HT4P shares 61% and 68% identity with the human and pig SAS proteins, respectively. HT4P shares several features characteristic of TM4FS proteins. The four conserved hydrophobic, transmembrane domains are T1 (K10-G34), T2 (V48-Q72), T3 (V73-A98) and T4 (F168-R191). T1 contains the highly conserved asparagine residue, N17, and T3 and T4 contain conserved residues at Q89 and E180, respectively. The major hydrophilic, extracellular domain in HT4P is located approximately between residues N100 and R167. This region of HT4P is the most divergent in terms of sequence similarity to the human SAS which suggests a different ligand. Several cysteine residues located in this region are conserved and are believed to be important for correct folding of this domain. The two N-linked glycosylation sites of HT4P are located in the hydrophilic domain. The potential casein kinase II phosphorylation site in HT4P at S177 is shared by the human SAS. As illustrated by Figures 3A, 3B, and 3C, HT4P and the human and pig SAS proteins have rather similar hydrophobicity plots. In particular, the four hydrophobic, transmembrane domains of HT4P are centered approximately

at residues 30, 60, 90, and 190. The major hydrophilic, extracellular domain of HT4P is centered approximately at residue 140. Northern analysis shows the expression of HT4P in various libraries, 41% of which are associated with cancer and immortalized cell lines, 33% of which are associated with smooth muscle and the sympathetic nervous system, and 18% of which are associated with the brain and neural tissues.

The invention also encompasses HT4P variants. A preferred HT4P variant is one having at least 80%, and more preferably 90%, amino acid sequence identity to the HT4P amino acid sequence (SEQ ID NO:1). A most preferred HT4P variant is one having at least 95% amino acid sequence identity to SEQ ID NO:1.

The invention also encompasses polynucleotide which encode HT4P. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of HT4P can be used to generate recombinant molecules which express HT4P. In a particular embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figures 1A, 1B and 1C.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding HT4P, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HT4P, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HT4P and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HT4P under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HT4P or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HT4P and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences, or portions thereof,

which encode HT4P and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HT4P or any portion thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency.

Altered nucleic acid sequences encoding HT4P which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent HT4P. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HT4P. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of HT4P is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

Also included within the scope of the present invention are alleles of the genes encoding HT4P. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp,

Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (Perkin Elmer).

The nucleic acid sequences encoding HT4P may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk in genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to

include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HT4P, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of HT4P in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express HT4P.

As will be understood by those of skill in the art, it may be advantageous to produce HT4P-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HT4P encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation

patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HT4P may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of HT4P activity, it may be useful to encode a chimeric HT4P protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the HT4P encoding sequence and the heterologous protein sequence, so that HT4P may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding HT4P may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of HT4P, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of HT4P, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active HT4P, the nucleotide sequences encoding HT4P or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HT4P and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John

Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HT4P. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding HT4P, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for HT4P. For example, when large quantities of HT4P are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding HT4P may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the

presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding HT4P may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express HT4P. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding HT4P may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of HT4P will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which HT4P may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HT4P may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing HT4P in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression

in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HT4P. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding HT4P, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express HT4P may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell

22:817-23) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, ß glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding HT4P is inserted within a marker gene sequence, recombinant cells containing sequences encoding HT4P can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HT4P under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding HT4P and express HT4P may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding HT4P can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotides encoding HT4P. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding HT4P to detect transformants containing DNA or RNA encoding HT4P. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of HT4P, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HT4P is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HT4P include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HT4P, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HT4P may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HT4P may be designed to contain signal sequences which direct secretion of HT4P through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding HT4P to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of

cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and HT4P may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing HT4P and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3:263-281) while the enterokinase cleavage site provides a means for purifying HT4P from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

In addition to recombinant production, fragments of HT4P may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of HT4P may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

THERAPEUTICS

Chemical and structural homology exists among HT4P, human SAS and pig SAS. In addition, northern analysis shows the expression of HT4P in tissues associated with cancer, smooth muscle, and neural tissues. HT4P therefore appears to be associated with the development of cancer, smooth muscle disorders, and neurological disorders.

In particular, a decrease in the level or activity of HT4P appears to be associated with the development of neurological disorders. Therefore in one embodiment, HT4P or a fragment or derivative thereof, may be administered to a subject to treat or prevent a neurological disorder. Such disorders may include, but are not limited to, akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, schizophrenia, and Tourette's disorder.

In another embodiment, a vector capable of expressing HT4P, or a fragment or a derivative thereof, may also be administered to a subject to treat any of the neurological disorders listed above.

Decreased expression of HT4P also appears to be associated with the development of smooth muscle disorders. Therefore in another embodiment, HT4P or a fragment or derivative

thereof, may be administered to a subject to treat or prevent a smooth muscle disorder. A smooth muscle disorder is defined as any impairment or alteration in the normal action of smooth muscle and may include, but is not limited to, angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma, and myopathies including cardiomyopathy, encephalopathy, epilepsy, Kearns-Sayre syndrome, lactic acidosis, myoclonic disorder, and ophthalmoplegia. Smooth muscle includes, but is not limited to, that of the blood vessels, gastrointestinal tract, heart, and uterus.

In another embodiment, a vector capable of expressing HT4P, or a fragment or a derivative thereof, may also be administered to a subject to treat any of the smooth muscle disorders listed above.

An increase in the level or activity of HT4P appears to be associated with the development of cancer. Therefore in another embodiment, an antagonist or inhibitor of HT4P may be administered to a subject to treat or prevent cancer, including astrocytoma, glioma, ganglioneuroma, neurocytoma, neuroblastoma. adenocarcinoma, sarcoma, melanoma, lymphoma, leukemia, and myeloma. In particular, types of cancer may include, but are not limited to, cancer of the adrenal gland, bladder, bone, brain, breast, gastrointestinal tract, heart, kidney, liver, lung, ovary, pancreas, paraganglia, parathyroid, prostate, salivary glands, skin, spleen, testis, thyroid, and uterus. In one aspect of this embodiment, antibodies which are specific for HT4P may be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HT4P.

In another embodiment, the complement of the polynucleotide encoding HT4P or an antisense molecule may be administered to a subject to treat or prevent any of the types of cancer listed above.

In other embodiments, any of the therapeutic proteins, antagonists, antibodies, agonists, antisense sequences or vectors of this invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Antagonists or inhibitors of HT4P may be produced using methods which are generally

known in the art. In particular, purified HT4P may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HT4P.

The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with HT4P or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies to HT4P have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HT4P amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to HT4P may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HT4P-specific single chain

antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for HT4P may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HT4P and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HT4P epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding HT4P, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding HT4P may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HT4P. Thus, antisense molecules may be used to modulate HT4P activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding HT4P.

Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense molecules complementary to the polynucleotides of the gene encoding HT4P. These techniques are described both in

Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding HT4P can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes HT4P. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA, or PNA, to the control regions of the gene encoding HT4P, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HT4P.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for

chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HT4P. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HT4P, antibodies to HT4P, mimetics, agonists, antagonists, or inhibitors of HT4P. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial,

intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HT4P, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HT4P or fragments thereof, antibodies of HT4P, agonists, antagonists or inhibitors of HT4P, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HT4P may be used for the diagnosis of conditions or diseases characterized by expression of HT4P, or in assays to monitor patients being treated with HT4P, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for HT4P include methods which utilize the antibody and a label to detect HT4P in human body fluids or extracts of cells or tissues. The antibodies may be used

with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring HT4P are known in the art and provide a basis for diagnosing altered or abnormal levels of HT4P expression. Normal or standard values for HT4P expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HT4P under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric, means. Quantities of HT4P expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HT4P may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of HT4P may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of HT4P, and to monitor regulation of HT4P levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HT4P or closely related molecules, may be used to identify nucleic acid sequences which encode HT4P. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding HT4P, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the HT4P encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring HT4P.

Means for producing specific hybridization probes for DNAs encoding HT4P include the

cloning of nucleic acid sequences encoding HT4P or HT4P derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as 32P or 35S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HT4P may be used for the diagnosis of conditions or diseases which are associated with expression of HT4P. Examples of such conditions or diseases include neurological disorders such as akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, schizophrenia, and Tourette's disorder; smooth muscle disorders such as angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma, and myopathies including cardiomyopathy, encephalopathy, epilepsy, Kearns-Sayre syndrome, lactic acidosis, myoclonic disorder, and ophthalmoplegia; and cancer such as cancer of the adrenal gland, bladder, bone, brain, breast, gastrointestinal tract, heart, kidney, liver, lung, ovary, pancreas, paraganglia, parathyroid, prostate, salivary glands, skin, spleen, testis, thyroid, and uterus. The polynucleotide sequences encoding HT4P may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered HT4P expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HT4P may be useful in assays that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding HT4P may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding HT4P in the sample indicates the presence of the associated

disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of HT4P, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes HT4P, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HT4P may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'->3') and another with antisense (3'<-5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HT4P include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard

curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In another embodiment of the invention, the nucleic acid sequences which encode HT4P may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) Blood Rev. 7:127-134, and Trask, B.J. (1991) Trends Genet. 7:149-154.

FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding HT4P on a physical chromosomal map and a specific disease , or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, HT4P, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between HT4P and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to HT4P large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with HT4P, or fragments thereof, and washed. Bound HT4P is then detected by methods well known in the art. Purified HT4P can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HT4P specifically compete with a test compound for binding HT4P. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HT4P.

In additional embodiments, the nucleotide sequences which encode HT4P may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

INDUSTRIAL APPLICABILITY

I PROSNON01 cDNA Library Construction

PROSNON01 is a normalized library constructed from 4.4 million independent clones from the PROSNOT11 cDNA library. The PROSNOT11 cDNA library was constructed from microscopically normal prostate tissue obtained from a 28-year-old Caucasian male (specimen #RA95-09-0677; International Institute of Advanced Medicine, Exton, PA) who died from a self inflicted gun shot wound.

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury NJ) in guanidinium isothiocyanate solution. The

lysate was centrifuged over a 5.7 M CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.7, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in RNAse-free water and DNase treated at 37°C. RNA extraction and precipitation were repeated as before. The mRNA was then isolated with the Qiagen Oligotex kit (QIAGEN, Chatsworth, CA) and used to construct the cDNA library. The normalization and hybridization conditions were adapted from Soares et al., PNAS 91 (1994):9228, except that a longer (19-hour) reannealing hybridization was used.

The mRNA was handled according to the recommended protocols in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (Cat. #18248-013, Gibco/BRL, Gaithersburg, MD). cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105-01, Pharmacia), and those cDNAs exceeding 400 bp were ligated into pSport I. The plasmid pSport I was subsequently transformed into DH5a™ competent cells (Cat. #18258-012, Gibco/BRL).

II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 Plasmid Kit (Catalog #26173, QIAGEN, Inc.). This kit enabled the simultaneous purification of 96 samples in a 96-well block using multi-channel reagent dispensers. The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711,Gibco/BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems; and the reading frame was determined.

III Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences of the Sequence Listing or amino acid sequences deduced from them were used as query sequences against databases such as GenBank, SwissProt, BLOCKS, and Pima II. These databases which contain previously identified and annotated sequences were searched for regions of homology (similarity) using BLAST, which stands for Basic Local Alignment Search Tool (Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul et al. (1990) J.

Mol. Biol. 215:403-410).

BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal or plant) origin. Other algorithms such as the one described in Smith RF and TF Smith (1992; Protein Engineering 5:35-51), incorporated herein by reference, can be used when dealing with primary sequence patterns and secondary structure gap penalties. As disclosed in this application, the sequences have lengths of at least 49 nucleotides, and no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach, as detailed in Karlin, S. and S.F. Atschul (1993; Proc. Nat. Acad. Sci. 90:5873-7) and incorporated herein by reference, searches for matches between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-14} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and mammalian sequences (mam), and deduced amino acid sequences from the same clones are searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp) and eukaryote (eukp), for homology. The relevant database for a particular match were reported as a GIxxx±p (where xxx is pri, rod, etc and if present, p = peptide).

IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact

within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding HT4P occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V Extension of HT4P-Encoding Polynucleotides

Nucleic acid sequence of Incyte Clone 2279874 or SEQ ID NO:2 is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary or desired, additional sets of primers are designed to further extend the known region.

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles

Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5-10 μl aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products are selected and removed from the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc., Chatsworth, CA). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μl of ligation buffer, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent E. coli cells (in 40 μl of appropriate media) are transformed with 3 μl of ligation mixture and cultured in 80 μl of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150 μl of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μl of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μl of each sample is transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2-4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid, and sequenced.

VI Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10^7 counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba 1, or Pvu II; DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

VII Complementary Polynucleotide, Antisense Molecules

Polynucleotide complementary to the HT4P-encoding sequence, or any part thereof, or an antisense molecule is used to inhibit in vivo expression of naturally occurring HT4P. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide based on the coding sequences of HT4P, as shown in Figures 1A, 1B and 1C, is used to inhibit expression of naturally occurring HT4P. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A, 1B and 1C and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an HT4P-encoding transcript by preventing the ribosome from binding. Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:2, an effective antisense

oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or 5' coding sequence of the polypeptide as shown in Figures 1A, 1B and 1C.

VIII Expression of HT4P

Expression of HT4P is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector is used to express HT4P in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent seven residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of ß-galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of HT4P into the bacterial growth media which can be used directly in the following assay for activity.

IX Demonstration of HT4P Activity

Given the chemical and structural similarity between HT4P protein and the human and pig SAS proteins, human HT4P is identified as a member of the TM4SF and is presumed to be involved in the regulation of cell growth. HT4P is expressed in various human carcinomas (prostate, lung, and breast), malignant growths that consist of epithelial cells that tend to infiltrate surrounding tissues and give rise to metastases. HT4P is also expressed in lung and brain tumor metastases and thus, expression of HT4P is presumed to be related to metastatic potential.

To demonstrate that increased levels of HT4P expression correlates with an increase in metastatic potential or increased cell motility, expression vectors encoding HT4P are transfected (e.g., electroporated) into non-motile or poorly motile cell lines (the majority of cell lines including cancer cell lines are non- or poorly motile), and the motility of the transfected and untransfected (control) cells are compared. Methods for the design and construction of an expression vector capable of expressing HT4P in the desired mammalian cell line(s) chosen are well known to the art. Assays for examining the motility of cells in culture are known to the art (Miyake, M. et al. (1991) J. Exp. Med. 174:1347-54; Ikeyama, S. et al. (1993), supra). If increasing the level of HT4P in non- or poorly motile cell lines by transfection with a HT4P expression vector increases the motility of the cell lines, then the level of HT4P expression correlates with cell motility and/or metastatic potential.

In addition to the assay described above, the correlation between HT4P and metastatic potential can be measured using an animal model in which the invasiveness of a non-metastatic

cell line such as the mouse B16B1 melanoma, transfected with HT4P is compared with non-transfected B16B1 and with the highly metastatic BL6 cell line (Ikeyama, et al., supra). Metastatic deposition in the lung is measured following intravenous injections of cells in Balb/c mice. An increase in lung deposition in the HT4P-transfected B16B1cells relative to the non-transfected B16B1 that approaches the metastasis produced by BL6 cells is evidence that levels of HT4P in tumor cells correlates with metastatic potential.

X Production of HT4P Specific Antibodies

HT4P that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopolypeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

XI Purification of Naturally Occurring HT4P Using Specific Antibodies

Naturally occurring or recombinant HT4P is substantially purified by immunoaffinity chromatography using antibodies specific for HT4P. An immunoaffinity column is constructed by covalently coupling HT4P antibody to an activated chromatographic resin, such as CnBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HT4P is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HT4P (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HT4P binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HT4P is collected.

XII Identification of Molecules Which Interact with HT4P

HT4P or biologically active fragments thereof are labeled with ^{125}I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133:529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HT4P, washed and any wells with labeled HT4P complex are assayed. Data obtained using different concentrations of HT4P are used to calculate values for the number, affinity, and association of HT4P with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: NEW HUMAN TRANSMEMBRANE PROTEIN

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) PCT APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/855,519
(B) FILING DATE: 13-MAY-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Billings, Lucy J.
(B) REGISTRATION NUMBER: 36,749
(C) REFERENCE/DOCKET NUMBER: PF-0292 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-855-0555
(B) TELEFAX: 650-845-4166
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 204 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: PROSNON01
(B) CLONE: 2279874

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Val Cys Gly Gly Phe Ala Cys Ser Lys Asn Cys Leu Cys Ala Leu
 1               5                  10                  15
```

```
Asn Leu Leu Tyr Thr Leu Val Ser Leu Leu Leu Ile Gly Ile Ala Ala
            20                  25                  30
Trp Gly Ile Gly Phe Gly Leu Ile Ser Ser Leu Arg Val Val Gly Val
        35                  40                  45
Val Ile Ala Val Gly Ile Phe Leu Phe Leu Ile Ala Leu Val Gly Leu
    50                  55                  60
Ile Gly Ala Val Lys His His Gln Val Leu Leu Phe Phe Tyr Met Ile
65                  70                  75                  80
Ile Leu Leu Leu Val Phe Ile Val Gln Phe Ser Val Ser Cys Ala Cys
                85                  90                  95
Leu Ala Leu Asn Gln Glu Gln Gln Gly Gln Leu Leu Glu Val Gly Trp
            100                 105                 110
Asn Asn Thr Ala Ser Ala Arg Asn Asp Ile Gln Arg Asn Leu Asn Cys
        115                 120                 125
Cys Gly Phe Arg Ser Val Asn Pro Asn Asp Thr Cys Leu Ala Ser Cys
    130                 135                 140
Val Lys Ser Asp His Ser Cys Ser Pro Cys Ala Pro Ile Ile Gly Glu
145                 150                 155                 160
Tyr Ala Gly Glu Val Leu Arg Phe Val Gly Gly Ile Gly Leu Phe Phe
                165                 170                 175
Ser Phe Thr Glu Ile Leu Gly Val Trp Leu Thr Tyr Arg Tyr Arg Asn
            180                 185                 190
Gln Lys Asp Pro Arg Ala Asn Pro Ser Ala Phe Leu
        195                 200
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1008 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: PROSNON01
(B) CLONE: 2279874

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
CACGTCTGCG TTGCTGCCCC GCCTGGGCCA GGCCCCAAAG GCAAGGACAA AGCAGCTGTC     60
AGGGAACCTC CGCCGGAGTC GAATTTACGT GCAGCTGCCG GCAACCACAG GTTCCAAGAT    120
GGTTTGCGGG GGCTTCGCGT GTTCCAAGAA CTGCCTGTGC GCCCTCAACC TGCTTTACAC    180
CTTGGTTAGT CTGCTGCTAA TTGGAATTGC TGCGTGGGGC ATTGGCTTCG GGCTGATTTC    240
CAGTCTCCGA GTGGTCGGCG TGGTCATTGC AGTGGGCATC TTCTTGTTCC TGATTGCTTT    300
AGTGGGTCTG ATTGGAGCTG TAAAACATCA TCAGGTGTTG CTATTCTTTT ATATGATTAT    360
TCTGTTACTT GTATTTATTG TTCAGTTTTC TGTATCTTGC GCTTGTTTAG CCCTGAACCA    420
GGAGCAACAG GGTCAGCTTC TGGAGGTTGG TTGGAACAAT ACGGCAAGTG CTCGAAATGA    480
CATCCAGAGA AATCTAAACT GCTGTGGGTT CCGAAGTGTT AACCCAAATG ACACCTGTCT    540
GGCTAGCTGT GTTAAAAGTG ACCACTCGTG CTCGCCATGT GCTCCAATCA TAGGAGAATA    600
TGCTGGAGAG GTTTTGAGAT TTGTTGGTGG CATTGGCCTG TTCTTCAGTT TTACAGAGAT    660
CCTGGGTGTT TGGCTGACCT ACAGATACAG GAACCAGAAA GACCCCCGCG CGAATCCTAG    720
TGCATTCCTT TGATGAGAAA ACAAGGAAGA TTTCCTTTCG TATTATGATC TTGTTCACTT    780
TCTGTAATTT TCTGTTAAGC TCCATTTGCC AGTTTAAGGA AGGAAACACT ATCTGGAAAA    840
GTACCTTATT GATAGTGGGA ATTATATATT TTTACTCTAT GTTTCTCTAC ATGTTTTTTT    900
CTTTCCGTTG CTGAAAAATA TTTGAAACTT GTGGTCTCTG AAGCTCGGTG GCACCTGGGA    960
ATTTACTGTA TTCATTGTCG GGCACTGTCC ACTGTGGCCT TTCTTAGG                 1008
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 210 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 457937

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Val Cys Gly Gly Phe Ala Cys Ser Lys Asn Ala Leu Cys Ala Leu
 1               5                  10                  15
Asn Val Val Tyr Met Leu Val Ser Leu Leu Leu Ile Gly Val Ala Ala
            20                  25                  30
Trp Gly Lys Gly Leu Gly Leu Val Ser Ser Ile His Ile Ile Gly Gly
        35                  40                  45
Val Ile Ala Val Gly Val Phe Leu Leu Leu Ile Ala Val Ala Gly Leu
    50                  55                  60
Val Gly Ala Val Asn His His Gln Val Leu Leu Phe Phe Tyr Met Ile
65                  70                  75                  80
Ile Leu Gly Leu Val Phe Ile Phe Gln Phe Val Ile Ser Cys Ser Cys
                85                  90                  95
Leu Ala Ile Asn Arg Ser Lys Gln Thr Asp Val Ile Asn Ala Ser Trp
            100                 105                 110
Trp Val Met Ser Asn Lys Thr Arg Asp Glu Leu Glu Arg Ser Phe Asp
        115                 120                 125
Cys Cys Gly Leu Phe Asn Leu Thr Thr Leu Tyr Gln Gln Asp Tyr Asp
    130                 135                 140
Phe Cys Thr Ala Ile Cys Lys Ser Gln Ser Pro Thr Cys Gln Met Cys
145                 150                 155                 160
Gly Glu Lys Phe Leu Lys His Ser Asp Glu Ala Leu Lys Ile Leu Gly
                165                 170                 175
Gly Val Gly Leu Phe Phe Ser Phe Thr Glu Ile Leu Gly Val Trp Leu
            180                 185                 190
Ala Met Arg Phe Arg Asn Gln Lys Asp Pro Arg Ala Asn Pro Ser Ala
        195                 200                 205
Phe Leu
    210
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 971980

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Asn Val Val Tyr Met Leu Val Gly Leu Leu Leu Ile Gly Val Ala Ala
            20                  25                  30
Trp Ala Lys Gly Leu Gly Leu Val Ser Ser Ile His Ile Ile Gly Gly
        35                  40                  45
Val Ile Ala Val Gly Val Phe Leu Leu Leu Ile Ala Val Ala Gly Leu
    50                  55                  60
```

```
Val Gly Ala Val Asn His His Gln Val Leu Leu Phe Phe Tyr Met Ile
65                  70                  75                  80
Ile Leu Gly Leu Val Phe Ile Phe Gln Phe Gly Ile Ser Cys Ser Cys
                85                  90                  95
Leu Ala Ile Asn Leu Ser Lys Gln Ala Gly Ile Ile Asn
            100                 105
```

What is claimed is:

1. A substantially purified human transmembrane 4 protein comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.

2. An isolated and purified polynucleotide sequence encoding the transmembrane 4 protein of claim 1.

3. A polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 2.

4. A hybridization probe comprising the polynucleotide sequence of claim 2.

5. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or variants thereof.

6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 2 or variants thereof.

7. A hybridization probe comprising the polynucleotide sequence of claim 6.

8. An expression vector containing the polynucleotide sequence of claim 2.

9. A host cell containing the vector of claim 8.

10. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 the method comprising the steps of:

a) culturing the host cell of claim 9 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

11. A pharmaceutical composition comprising a substantially purified transmembrane 4 protein having an amino acid sequence of claim 1 in conjunction with a suitable pharmaceutical carrier.

12. A purified antibody which binds specifically to the polypeptide of claim 1.

13. A purified agonist which modulates the activity of the polypeptide of claim 1.

14. A purified antagonist which decreases the activity of the polypeptide of claim 1.

15. A method for treating a neurological disorder comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 11.

16. A method for treating a smooth muscle disorder comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 11.

17. A method for treating cancer comprising administering to a subject in need of such treatment an effective amount of the purified antagonist of claim 14.

18. A method for detection of a polynucleotide encoding a transmembrane 4 protein in a biological sample comprising the steps of:

a) hybridizing the polynucleotide of claim 6 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding a transmembrane 4 protein in said biological sample.

```
          9          18          27          36          45          54
NNC ACG TCT GCG TTG CTG CCC CGC CTG GGC CAG GCC CCA AAG GCA AGG ACA AAG

         63          72          81          90          99         108
CAG CTG TCA GGG AAC CTC CGC CGG AGT CGA ATT TAC GTG CAG CTG CCG GCA ACC

        117         126         135         144         153         162
ACA GGT TCC AAG ATG GTT TGC GGG GGC TTC GCG TGT TCC AAG AAC TGC CTG TGC
              M   V   C   G   G   F   A   C   S   K   N   C   L   C

        171         180         189         198         207         216
GCC CTC AAC CTG CTT TAC ACC TTG GTT AGT CTG CTG CTA ATT GGA ATT GCT GCG
A   L   N   L   L   Y   T   L   V   S   L   L   L   I   G   I   A   A

        225         234         243         252         261         270
TGG GGC ATT GGC TTC GGG CTG ATT TCC AGT CTC CGA GTG GTC GGC GTG GTC ATT
W   G   I   G   F   G   L   I   S   S   L   R   V   V   G   V   V   I

        279         288         297         306         315         324
GCA GTG GGC ATC TTC TTG TTC CTG ATT GCT TTA GTG GGT CTG ATT GGA GCT GTA
A   V   G   I   F   L   F   L   I   A   L   V   G   L   I   G   A   V

      333         342         351         360         369         378
AAA CAT CAT CAG GTG TTG CTA TTC TTT TAT ATG ATT ATT CTG TTA CTT GTA TTT
K   H   H   Q   V   L   L   F   F   Y   M   I   I   L   L   L   V   F
```

FIGURE 1A

```
         387        396        405        414        423        432
ATT GTT CAG TTT TCT GTA TCT TGC GCT TGT TTA GCC CTG AAC CAG GAG CAA CAG
I   V   Q   F   S   V   S   C   A   C   L   A   L   N   Q   E   Q   Q

         441        450        459        468        477        486
GGT CAG CTT CTG GAG GTT GGT TGG AAC AAT ACG GCA AGT GCT CGA AAT GAC ATC
G   Q   L   L   E   V   G   W   N   N   T   A   S   A   R   N   D   I

         495        504        513        522        531        540
CAG AGA AAT CTA AAC TGC TGT GGG TTC CGA AGT GTT AAC CCA AAT GAC ACC TGT
Q   R   N   L   N   C   C   G   F   R   S   V   N   P   N   D   T   C

         549        558        567        576        585        594
CTG GCT AGC TGT GTT AAA AGT GAC CAC TCG TGC TCG CCA TGT GCT CCA ATC ATA
L   A   S   C   V   K   S   D   H   S   C   S   P   C   A   P   I   I

         603        612        621        630        639        648
GGA GAA TAT GCT GGA GAG GTT TTG AGA TTT GTT GGT GGC ATT GGC CTG TTC TTC
G   E   Y   A   G   E   V   L   R   F   V   G   G   I   G   L   F   F

         657        666        675        684        693        702
AGT TTT ACA GAG ATC CTG GGT GTT TGG CTG ACC TAC AGA TAC AGG AAC CAG AAA
S   F   T   E   I   L   G   V   W   L   T   Y   R   Y   R   N   Q   K

         711        720        729        738        747        756
GAC CCC CGC GCG AAT CCT AGT GCA TTC CTT TGA TGA GAA AAC AAG GAA GAT TTC
D   P   R   A   N   P   S   A   F   L
```

FIGURE 1B

```
         765          774          783          792          801          810
CTT TCG TAT TAT GAT CTT GTT CAC TTT CTG TAA TTT TCT GTT AAG CTC CAT TTG

         819          828          837          846          855          864
CCA GTT TAA GGA AGG AAA CAC TAT CTG GAA AAG TAC CTT ATT GAT AGT GGG AAT

         873          882          891          900          909          918
TAT ATA TTT TTA CTC TAT GTT TCT CTA CAT GTT TTT TTC TTT CCG TTG CTG AAA

         927          936          945          954          963          972
AAT ATT TGA AAC TTG TGG TCT CTG AAG CTC GGT GGC ACC TGG GAA TTT ACT GTA

         981          990          999         1008
TTC ATT GTC GGG CAC TGT CCA CTG TGG CCT TTC TTA GG
```

FIGURE 1C

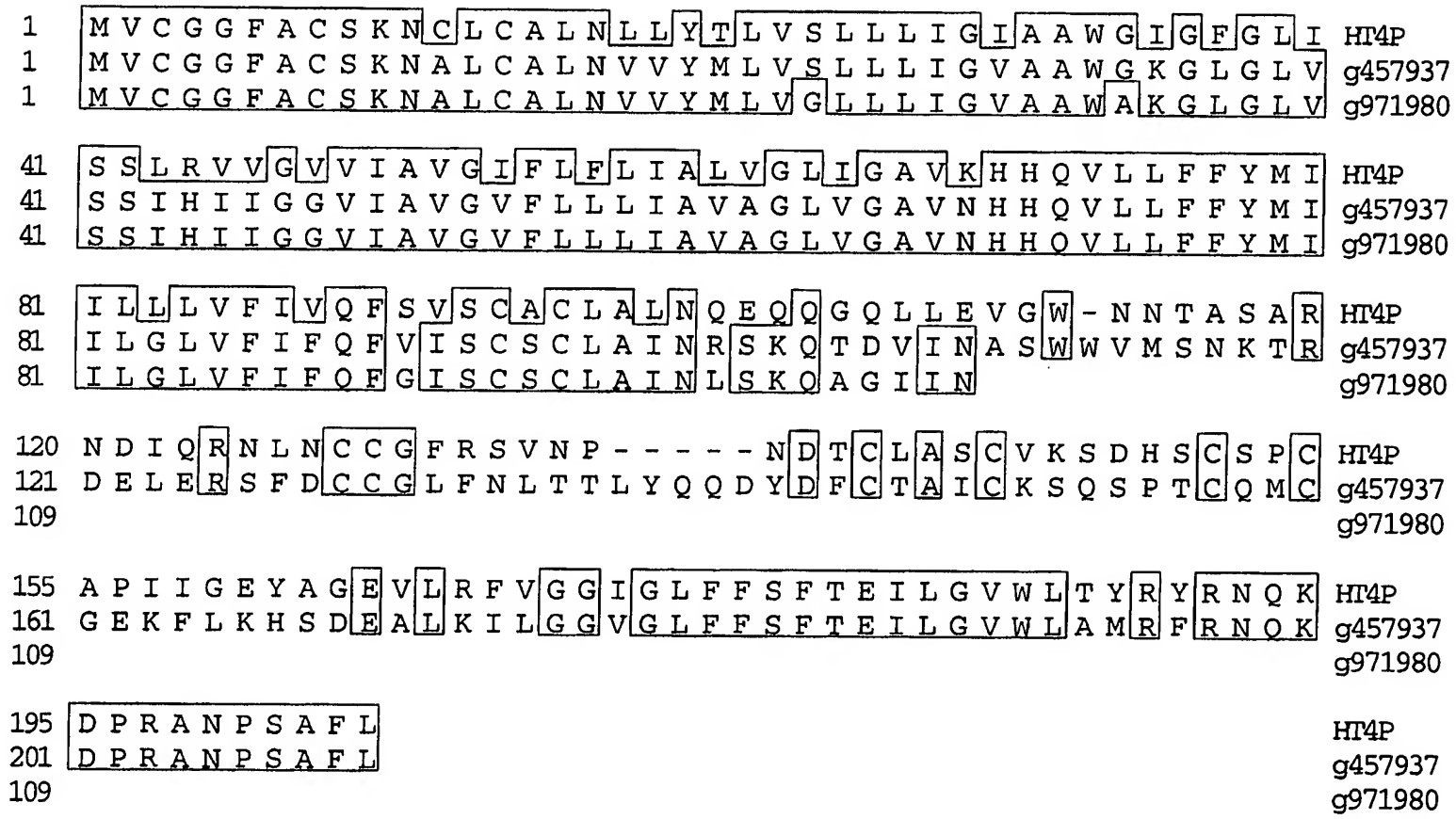
1 MVCGGFACSKNCLCALNLLYTLVSLLLIGIAAWGIGFGLI HT4P
1 MVCGGFACSKNALCALNVVYMLVSLLLIGVAAWGKGLGLV g457937
1 MVCGGFACSKNALCALNVVYMLVGLLLIGVAAWAKGLGLV g971980
41 SSLRVVGVVIAVGIFLFLIALVGLIGAVKHHQVLLFFYMI HT4P
41 SSIHIIGGVIAVGVFLLLIAVAGLVGAVNHHQVLLFFYMI g457937
41 SSIHIIGGVIAVGVFLLLIAVAGLVGAVNHHQVLLFFYMI g971980
81 ILLLVFIVQFSVSCACLALNQEQQGQLLEVGW-NNTASAR HT4P
81 ILGLVFIFQFVISCSCLAINRSKQTDVINASWWVMSNKTR g457937
81 ILGLVFIFQFGISCSCLAINLSKQAGIIN g971980
120 NDIQRNLNCCGFRSVNP-----NDTCLASCVKSDHSCSPC HT4P
121 DELERSFDCCGLFNLTTLYQQDYDFCTAICKSQSPTCQMC g457937
109 g971980
155 APIIGEYAGEVLRFVGGIGLFFSFTEILGVWLTYRYRNQK HT4P
161 GEKFLKHSDEALKILGGVGLFFSFTEILGVWLAMRFRNQK g457937
109 g971980
195 DPRANPSAFL HT4P
201 DPRANPSAFL g457937
109 g971980

FIGURE 2

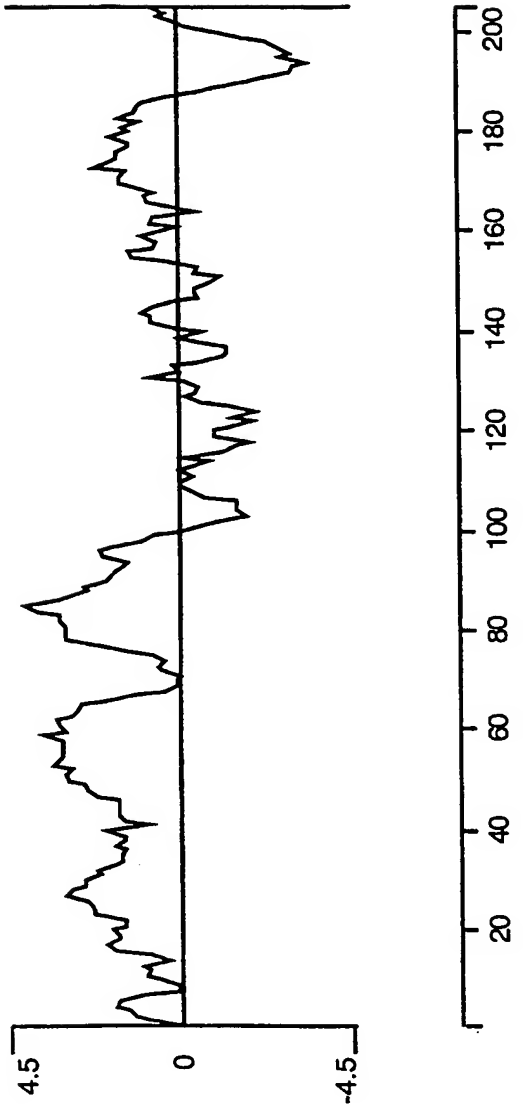

FIGURE3A

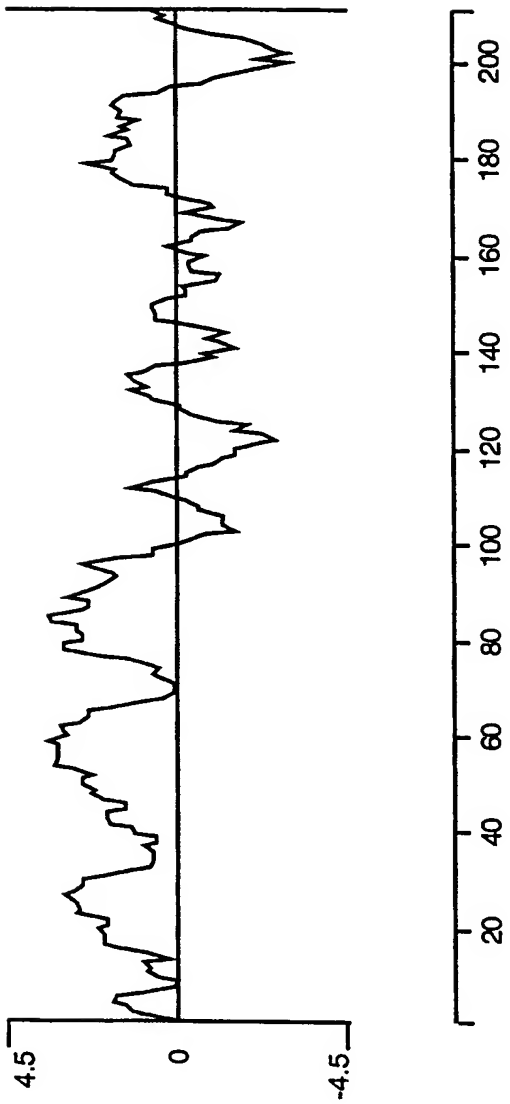

FIGURE3B

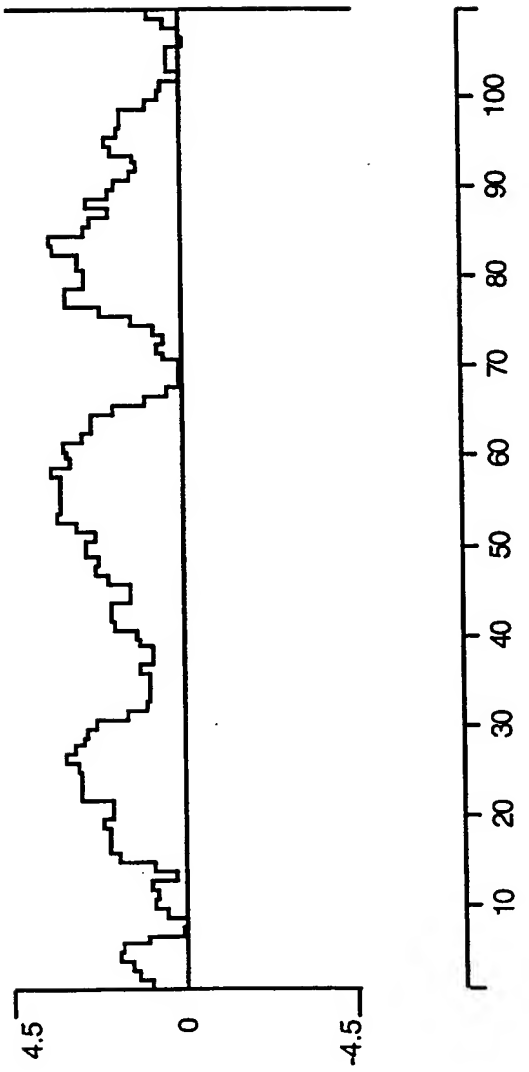

FIGURE 3C

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